

Creation of Designer Alga for Efficient and Robust Production of H₂

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Project ID #: PD17

Overview

Timeline

- Project start date: 08/2004
- Project end date 09/2008
- Percent complete 20%

Budget

- Total project funding
 - DOE share 100%
 - Contractor share
- Funding received in FY04: \$100K
- Funding for FY05: \$600K

Barriers

- Barriers addressed
 - J. Rate of Hydrogen Production. **The current hydrogen production rate from photosynthetic micro-organisms is far too low for commercial viability. Changes to these organisms, such as the genetic insertion of a proton channel into the thylakoid membrane, are required to overcome the restricting metabolic pathways to significantly increase the rate of hydrogen production.**

Partners

- University of Missouri-Columbia (D. Xu)
- University of Chicago (L. Mets)
- NREL (M. Ghirardi and M. Seibert) and UC Berkeley (T. Melis)

Objectives

Long-term objective:

Overcome nation's roadblocks to photosynthetic H₂ production through creation of designer alga by genetic insertion of a proton channel into algal thylakoid membrane—to solve the four proton gradient-related problems in algal H₂ production—to meet DOE H₂ Program goal (\$10/MMBtu).

FY05 objectives:

(1) Perform computer-assisted design of DNA sequence coding for a proton channel suitable for targeted insertion into algal thylakoid membrane (Task 1.4.1 described in the DOE-EERE/ORNL AOP)

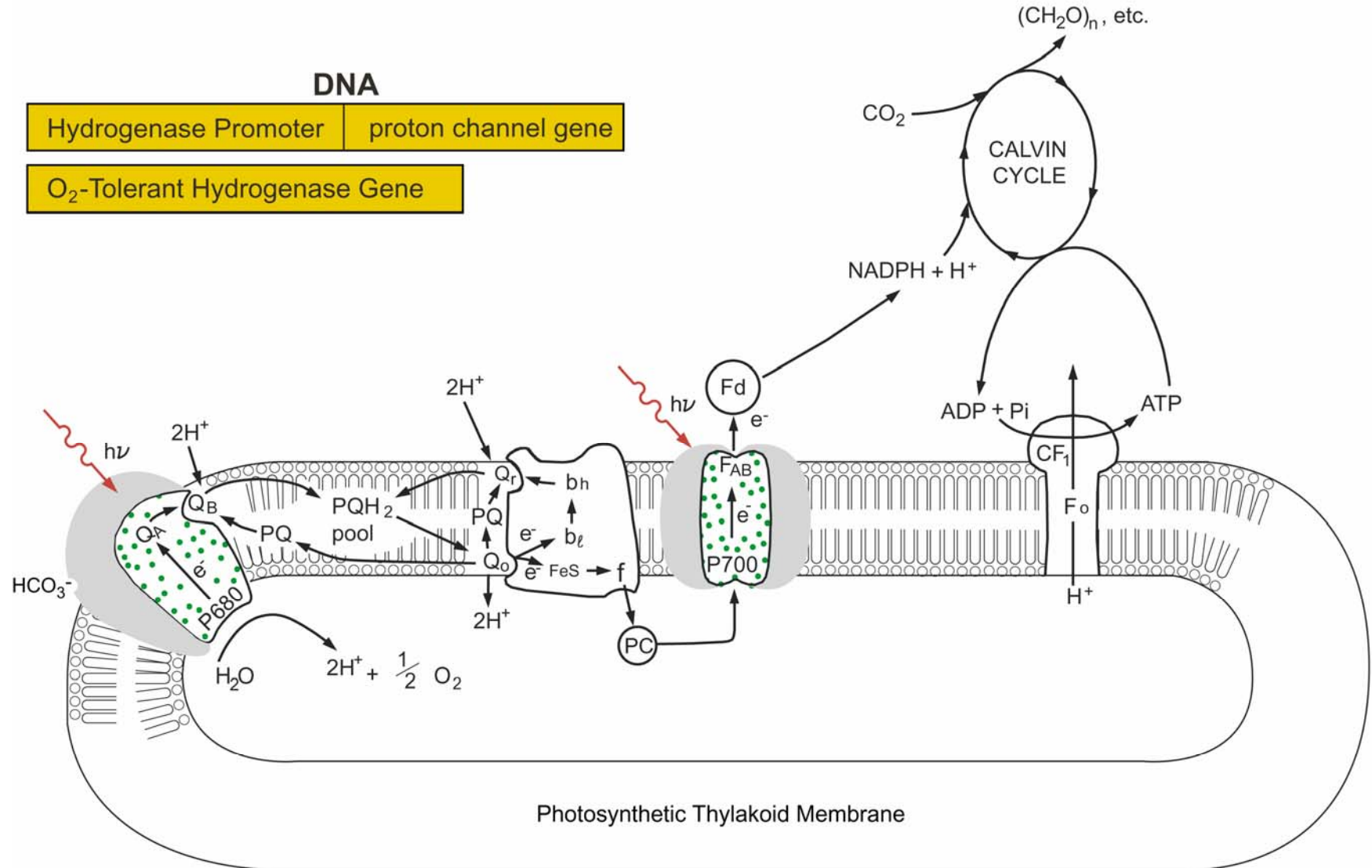
(2) Synthesize the proton-channel gene linked with hydrogenase promoter and thylakoid-signal-polypeptide DNA (Task 1.4.2 described in the DOE-EERE/ORNL AOP)

The ORNL algal H2 project will solve the first four problems (1–4) while NREL and UC Berkeley will solve problems 5 and 6

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- The diagram illustrates the flow of electrons and protons during photosynthesis. Light energy ($h\nu$) is absorbed by the Photosynthetic Thylakoid Membrane, which contains the Light-Harvesting Complex (LHC) and Photosystem II (P680). At P680, water (H_2O) is split into $2H^+$ and $\frac{1}{2} O_2$. Electrons (e^-) are transferred through a series of carriers: Q_A , Q_B , the Plastoquinone (PQ) pool, Q_C , Q_D , and the Plastocyanin (PC) complex. The electrons then reach Photosystem I (P700), where they are re-energized by light. From P700, electrons move through F_A , F_B , and finally to Ferredoxin (Fd). Ferredoxin donates electrons to NADP $^+$, which is reduced to NADPH. The resulting proton gradient across the membrane drives ATP synthesis by ATP synthase (CF $_1$ F $_o$), converting ADP and inorganic phosphate (Pi) into ATP. The Calvin Cycle, located in the stroma, uses NADPH and ATP to fix CO_2 (at 1000 ppm) into organic molecules like $(CH_2O)_n$. The cycle involves RuBisCo and RuDP. The diagram also highlights the competitive inhibition of RuBisCo by O_2 and the sensitivity of Hydrogenase to O_2 .

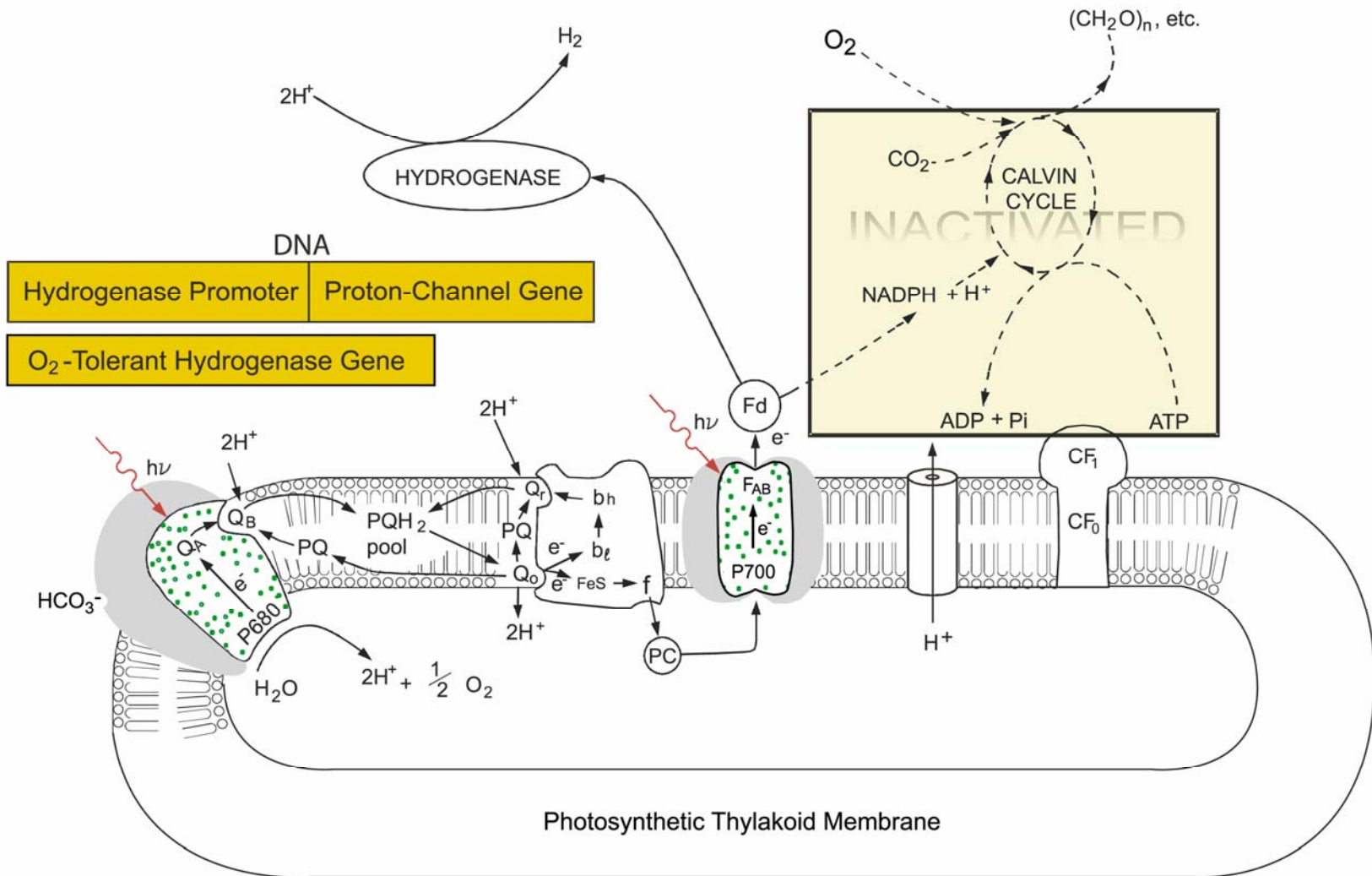
4

ORNL-Invented Concept: Designer Alga Performing Normal Photosynthesis under **Aerobic** Conditions



ORNL 2002-04253/vwp

Solution: Designer Alga Becomes an Efficient and Robust H₂-Production System under **Anaerobic** Conditions



ORNL 2002-04334/vwp

The ORNL Approach

To create switchable proton-channel designer alga through genetic insertion of proton channels into algal thylakoid membranes to simultaneously eliminate the four proton-gradient physiological problems that constitute the technical barrier “J. Rate of Hydrogen Production”:

- (1) Restriction of photosynthetic H₂ production by accumulation of a proton gradient;**
- (2) Competitive inhibition of photosynthetic H₂ production by CO₂;**
- (3) Requirement of bicarbonate binding at PSII for efficient photosynthetic activity; and**
- (4) Newly discovered O₂ sensitivity (drainage of electrons by O₂) in algal H₂ production.**

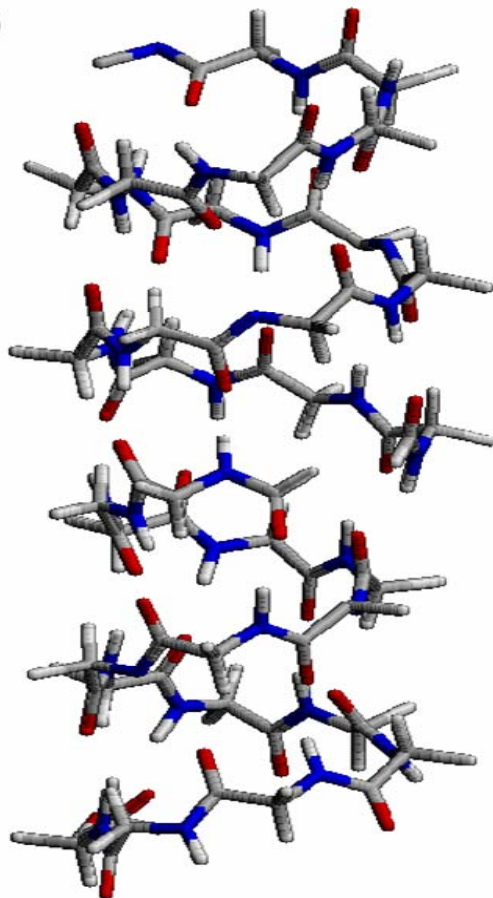
Technical Accomplishments/ Progress/Results

- Accomplished computer-assisted design of DNA sequences for the first set of the envisioned proton-channel genes;
- Synthesized the designed proton-channel genes linked with hydrogenase promoter and thylakoid-signal-polypeptide DNA.

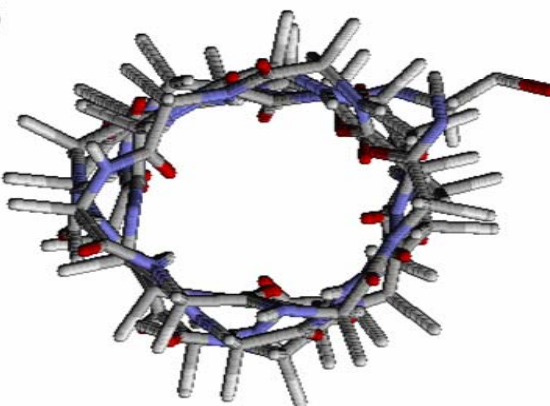
A Preliminary Design of Polypeptide Proton Channel Achieved by Computer Simulations at ORNL

ORNL 2002-02098/dgc

(a)



(b)



Accomplished: DNA Design for Synthetic Gene to Encode for a Proton Channel (gramicidin analog) in Algal Thylakoid Membrane

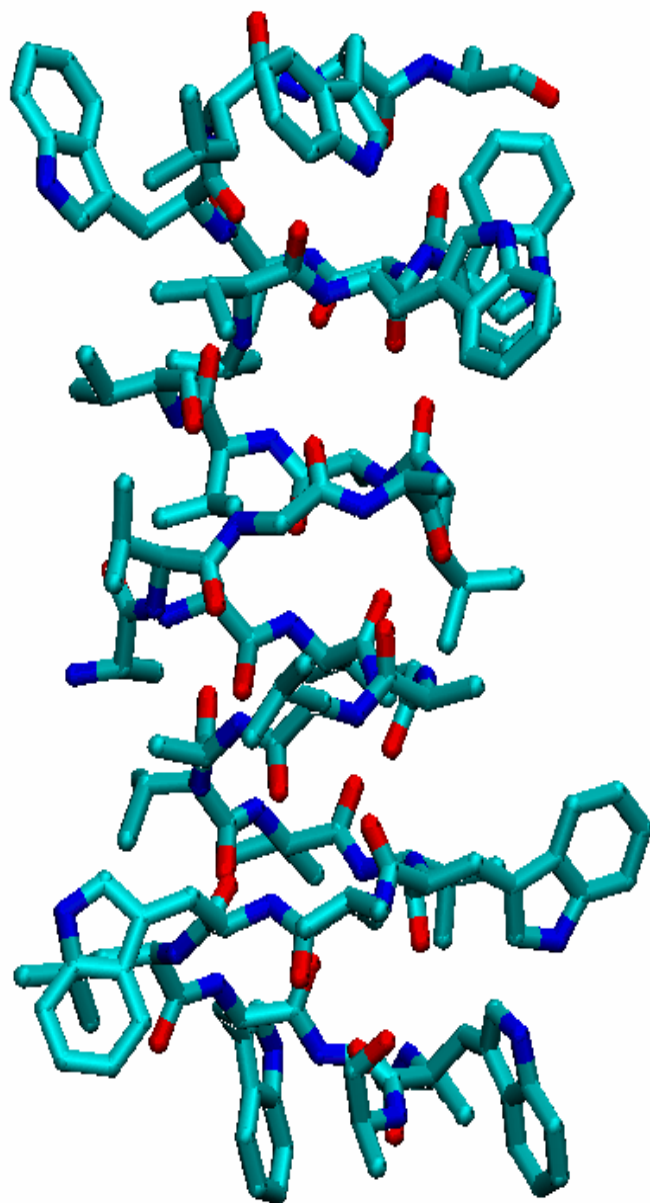
Design No. 1 for Expressing Gramicidin Analog

Hase promoter + *RbcS1* transit peptide + Gramicidin Analog + “natural” 3 UTR
Sequence: **570** bp

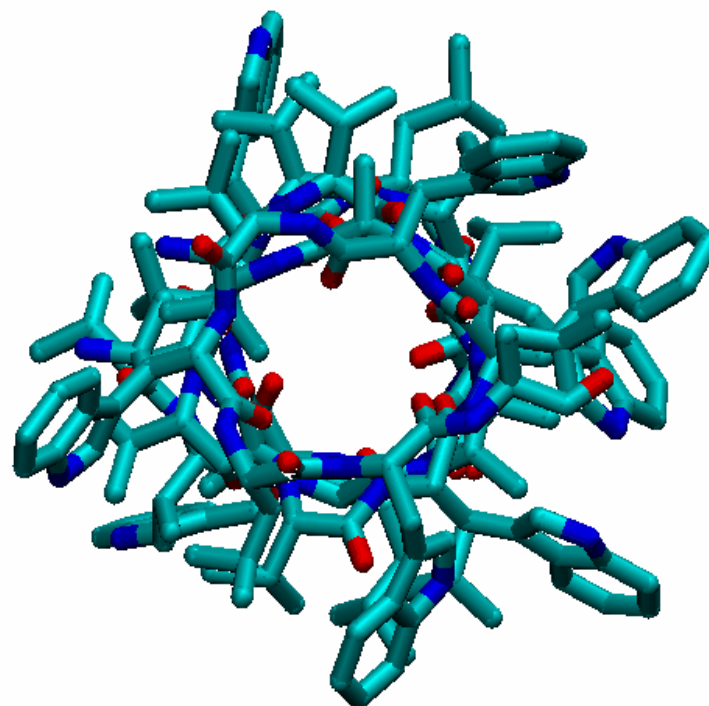
```
CCCACCGCTGTTTCTCCTGGATTTATGGATTTTATACTGGCATCTTTCAAGTCACGGAA
AAAGCGCGCGCTTCCGACGAAGGTAGGGCTGCACATGGCGAGACCTGCAGCTCAGCAT-
-----
CGTTCTCATTCCGCCATTCCTACTGGCGCCTTTAAATGGCAGGACCGCATCCAAGCTTAA
ACAATCTGTTCAAATATACAAGTGCcatATGGCCGCCGTCATTGCCAAGTCCTCCGTCTCC
-----
GCGTCAAGGCTGCCCCCGTGGCTGCCCCGGCTCAGGCCAACCAGGCCGTGGGCGCCCTG
-----
GTGGCTGTGGCTGTGGGCCTAAGCAGTTGACATGTTTTGG-----
ATGTAACATCCCGTGTGCA---
```

Our latest Design of Polypeptide Proton Channel Achieved by Computer Simulations
in collaboration with Prof. D. Xu

Side
View



Top View



Accomplished: DNA Sequence Design for Another Synthetic Gene to Encode for a Proton Channel (Melittin) in Algal Thylakoid Membrane

Design No. 2 for Expressing Melittin

Hase promoter + Plastocyanin transit peptide + Melittin + “natural” 3 UTR

Sequence: **603bp**

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CCCACCGCTCTTTCTCCTGGATTTATGGATTTTATACTGGCATCTTTCAAGTCACGGAA
AAAGCGCGCGCTTCCGACGAAGGTAGGGCTGCACATGGCGAGACCTGCAGCTCAGCAT-
-----
CGTTCTCATTCCGCCATTCCTACTGGCGCCTTTAAATGGCAGGACCGCATCCAAGCTTAA
ACAATCTGTTCAAATATACAAGTGCcatatgaaggctactctgcgtgccccgctcccgcgccagcgctgtgc-
-----
gccccgtcgccagcctgaaggccgctgctcagcgcggtggcctcggtcgccggtgtgtcggttgccctcttgccctgaccctggc
tgccacgcccGgcatcgggcgccgtcctg-----
aagegccagcagTAAGCAGTTGACAT-----
ATGTAACATCCCGTGTGCA---
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Completed the synthesizing of the first 3 designer proton-channel genes and ready for gene transformation



Reviewers' Comments

- Our reviewers clearly understood our proposed switchable-proton-channel designer alga H₂-production R&D concept. They commented that our approach is “very creative” and “addresses 4 barriers to biological production of H₂”.
- They further commented, our project employs an “integrated, well thought out approach” and “could produce a significant breakthrough in biological H₂ production.”
- “No cost breakdown or estimate; no attention to balance of plant or implementation”—Proof-of-principle (FCCP) experimental data demonstrated that use of this approach (genetic insertion of proton channel) could improve photobiological H₂ production rate by a factor of more than 10 times. More process economics analysis will follow if (or when) funding support allows.
- “Limited funding”—Thank the reviewers for recognizing this weakness; Hopefully the DOE H₂ Program could provide better funding support for the project.


Future Work

If the required 3.0-FTE project effort can be fully supported, we should be able to achieve the following milestones (tasks) in FY2006:

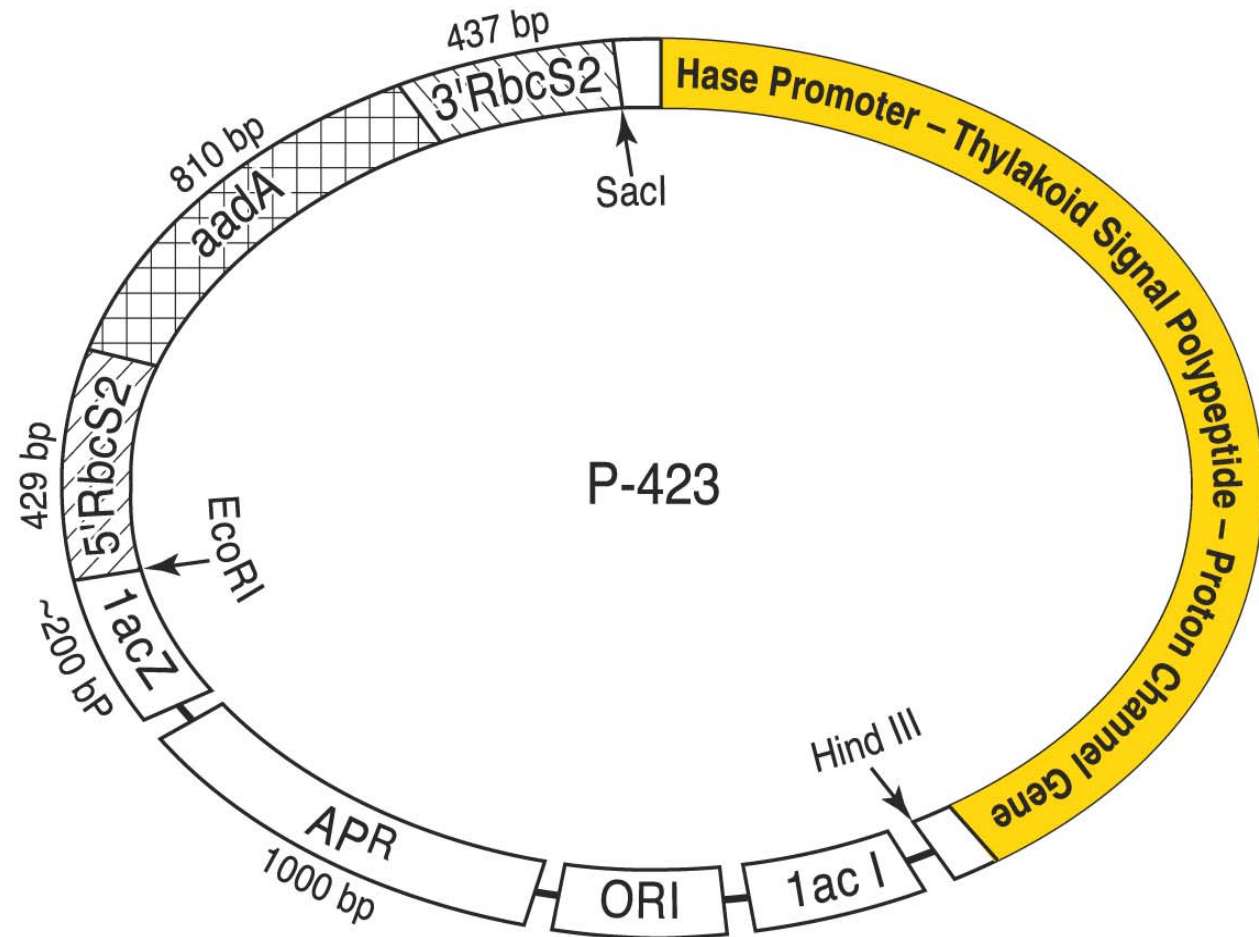
- Complete the assembly of the constructed hydrogenase promoter- thylakoid signal polypeptide-proton channel gene into a shuttle vector with a selectable marker for *Chlamydomonas reinhardtii* and *E. coli*.
- Accomplish propagation and verification of the DNA sequence for the synthetic hydrogenase promoter- thylakoid signal polypeptide-proton channel gene.
- Achieve genetic transfer of the first hydrogenase promoter-linked polypeptide proton-channel gene (DNA) into a host *Chlamydomonas reinhardtii* strain.

Use of Plasmid Vector for DNA Propagation and Analysis of Our Envisioned Synthetic Genes for Gene Transformation

ORNL 2002-02072b/vwp


ORNL will construct
(1st year project goal)


Commercially
available



We Can Deliver the Genes (DNA) into Our *Chlamydomonas* Host Cells
by Use of Electroporation or Glass-Beads Method



The Transformants will Be Screened and Cultured for a Number of Assays to Test for the Predicted Features of the Designer Alga



DNA Analyzers at ORNL

ORNL 2002-01800/vwp



**ABI PRISM 3700 DNA Analyzer
by Perkin-Elmer Applied Biosystems**

- DNA Sequencing and Fragment Analysis
- 96 Capillary Array allows for 100s of sequences a day
- Can sequence 550 base pairs with 98.5% accuracy
- Fragment Sizing within 0.5 bases up to 500 base pairs
- Automated sample loading, electrophoresis and data analysis



**HTS 7000 Plus BioAssay Reader by
Perkin-Elmer**

- DNA and Protein Quantitation
- Curve-fitting options provides tabular reports of quantitative and qualitative results
- High plate reading speeds (25 seconds/96 well plate)



iCycler Thermal Cycler by Bio-Rad

- Useful for accurate real-time quantitative PCR
- Capable of rapid temperature cycling, heating at a rate of up to 3.3 °C per second and cooling at a rate of up to 2.0 °C per second
- Highly accurate and uniform temperatures
- Real time, on-line displays enable visual confirmation of amplification success

Microarray Equipment for mRNA Assays at ORNL

ORNL 2002-01799/vwp



PixSys 5500XL by Cartesian Technologies

- High Throughput Arraying-Can prepare 48 microarrays at a time!
- 32 or 48 ChipMaker quill pins for multiple spotting from a single sample loading
- Vacuum wash station for cleaning between transfers
- Staker and Destacker (Holds 50 Plates)
- Humidity chamber for maintaining humidity and reducing dust



Scan Array 5000 by GSI Lumonics

- Compatible with many fluorescent dyes and labels
- (Cy2, Cy3, Cy5, FITC, TAMRA and more)
- Dye Choices can be combined to provide wide spectral spacing for 2, and 4 color applications
- Dye alternatives can provide higher sensitivity and signal variety



GeneTAC G3 Robotic Workstation by Genomic Solutions

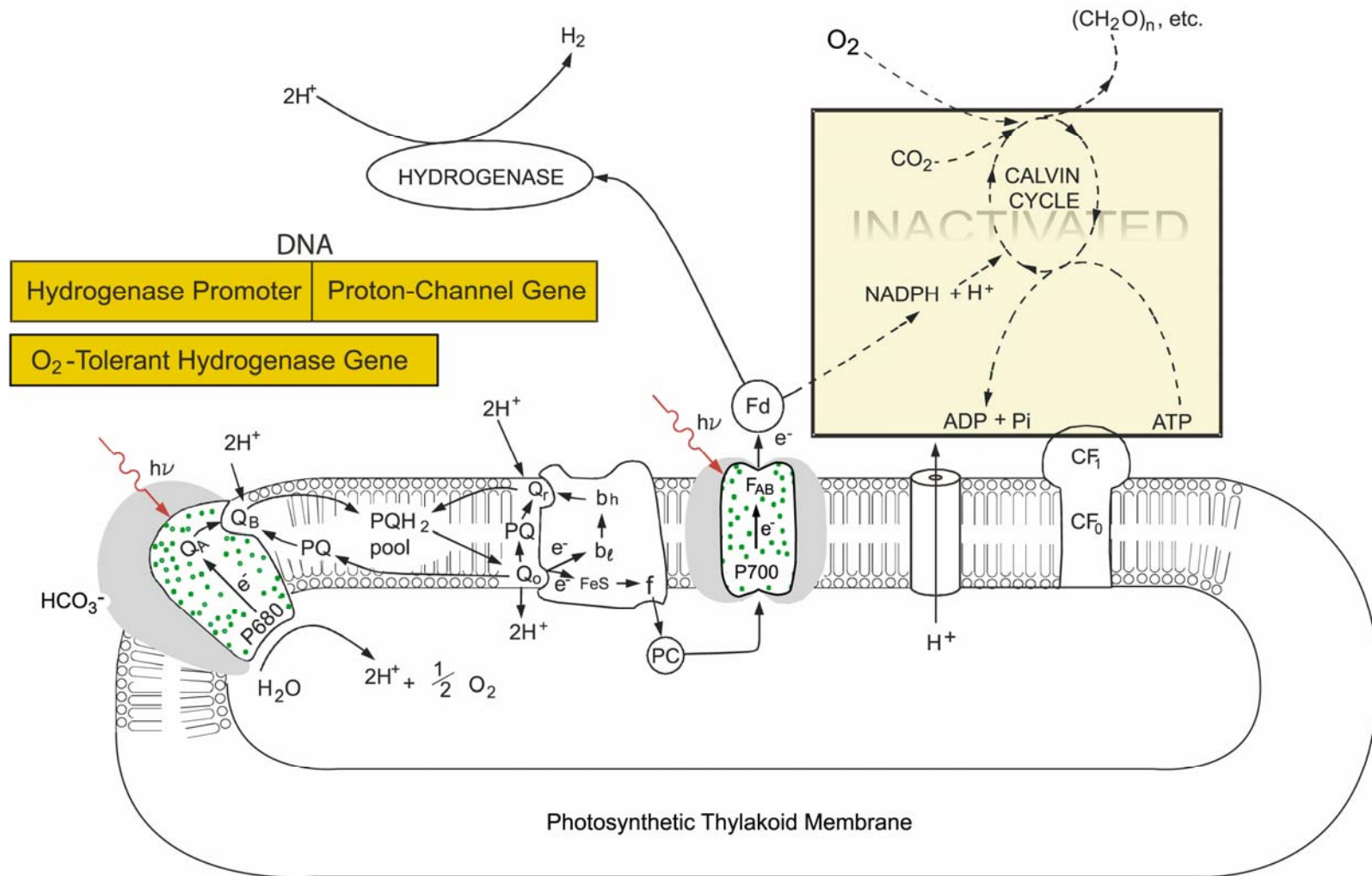
- For microarray production, library generation, and library management
- Gives flexibility for printing microarrays, colony picking, macroarraying, replication, and selective re-arraying
- Uses "dip and print" technique so that only 1 nl of sample is used - no wasted slides
- Pins are made from solid titanium



Our Customer-Designed State-of-the-Art Photospectrometer System
Can Be Used to Measure the Activity of the Envisioned Polypeptide-
Proton Channels in the Designer Alga at ORNL

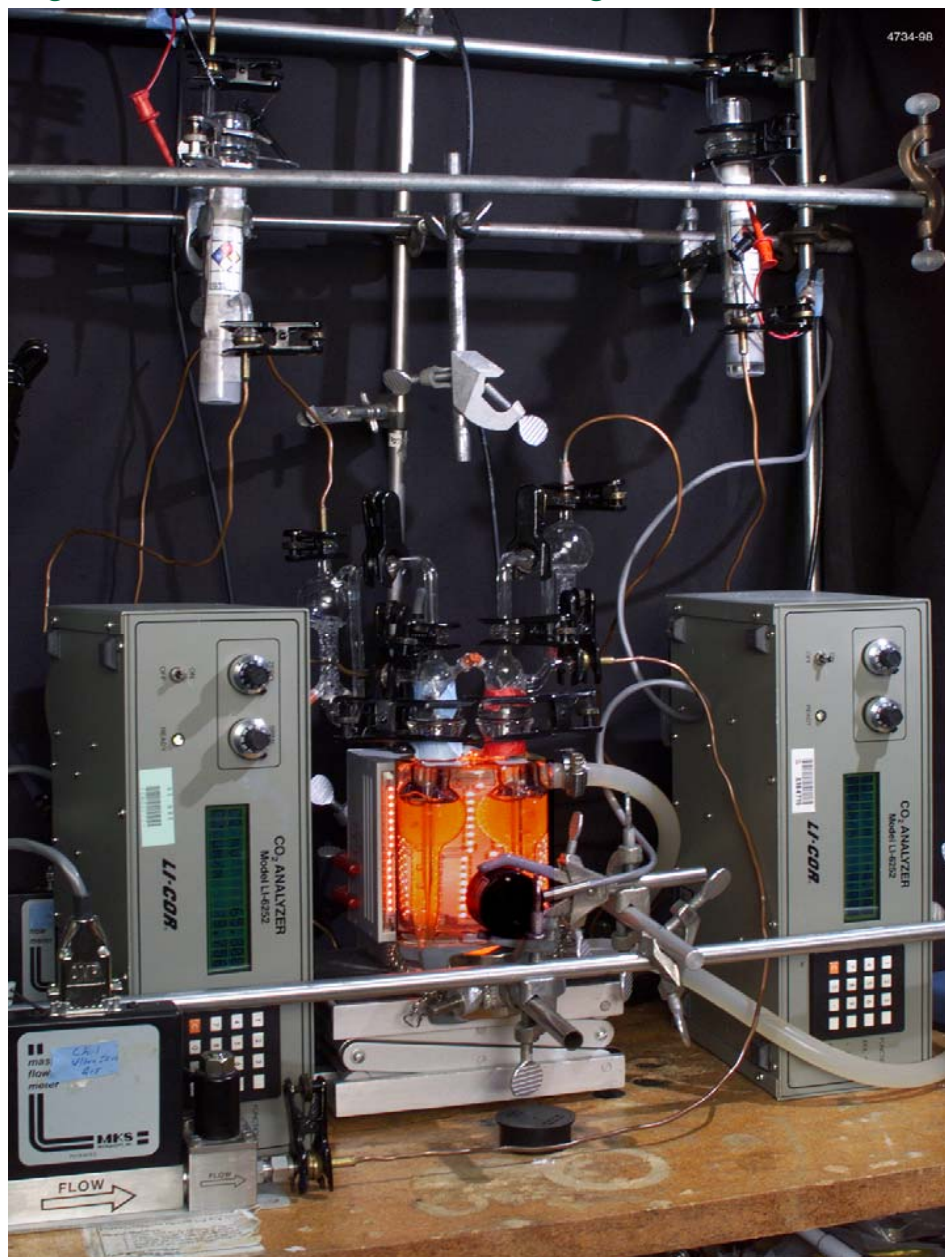


We Will Measure the Effects of the Polypeptide Proton Channels in Designer Alga through Photospectroscopic, Algal-Growth, and H₂-Production Assays

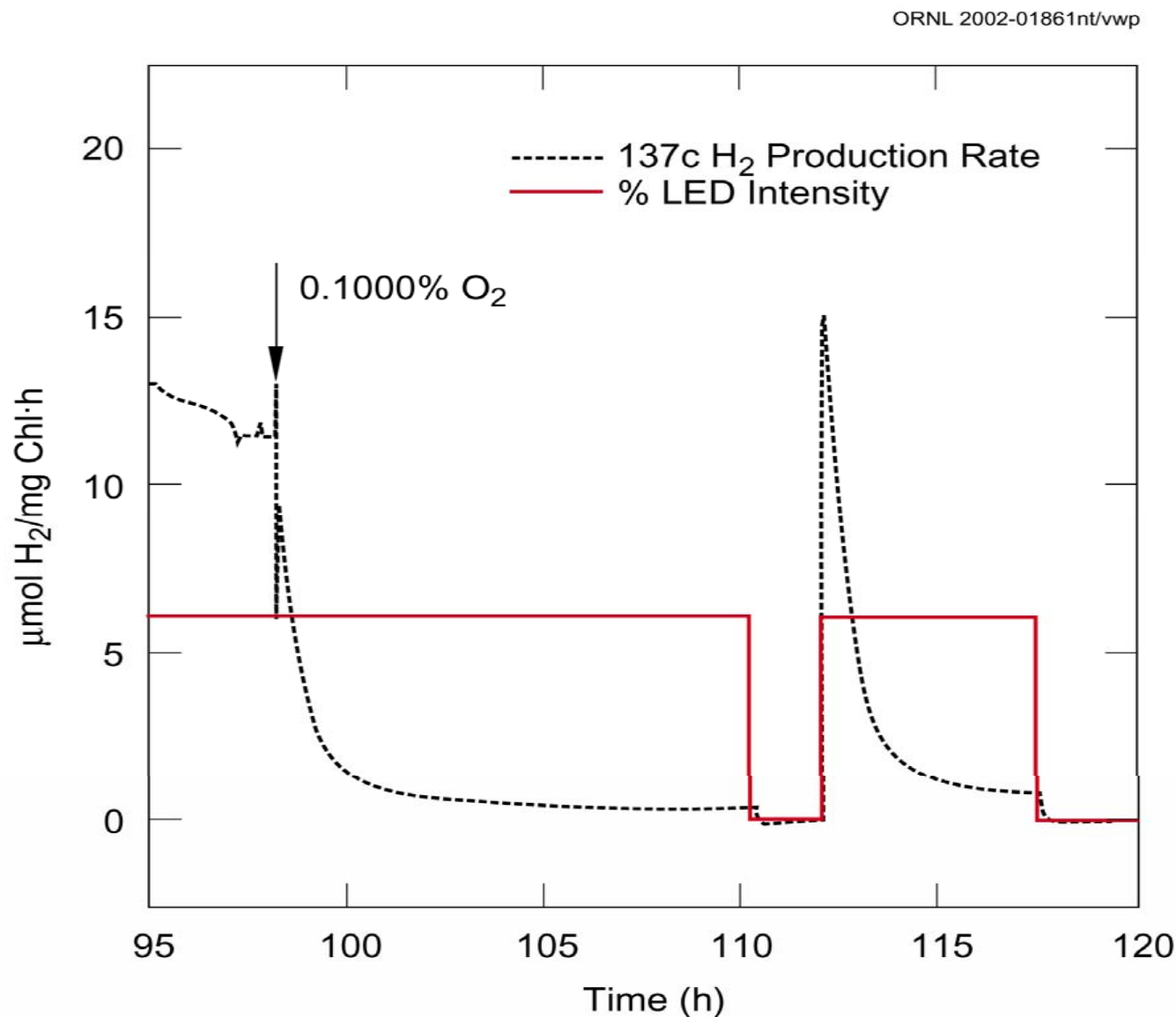


ORNL 2002-04252/vwp

Our Dual-Reactor-Flow Detection System Can Be Used for both H₂-Production and Recyclable-Growth Assays



Property of Our Newly Discovered O₂ Sensitivity in Wild-Type (*C. reinhardtii* 137c) Algal H₂ Production Can Be Used as a Reference to Test the Designer Alga



Path Forward - Milestones

Creation of designer alga for efficient and robust production of H₂
[3.0 FTE effort by Lee, Xu, Evans, Mets, Zhou, and Zhao]

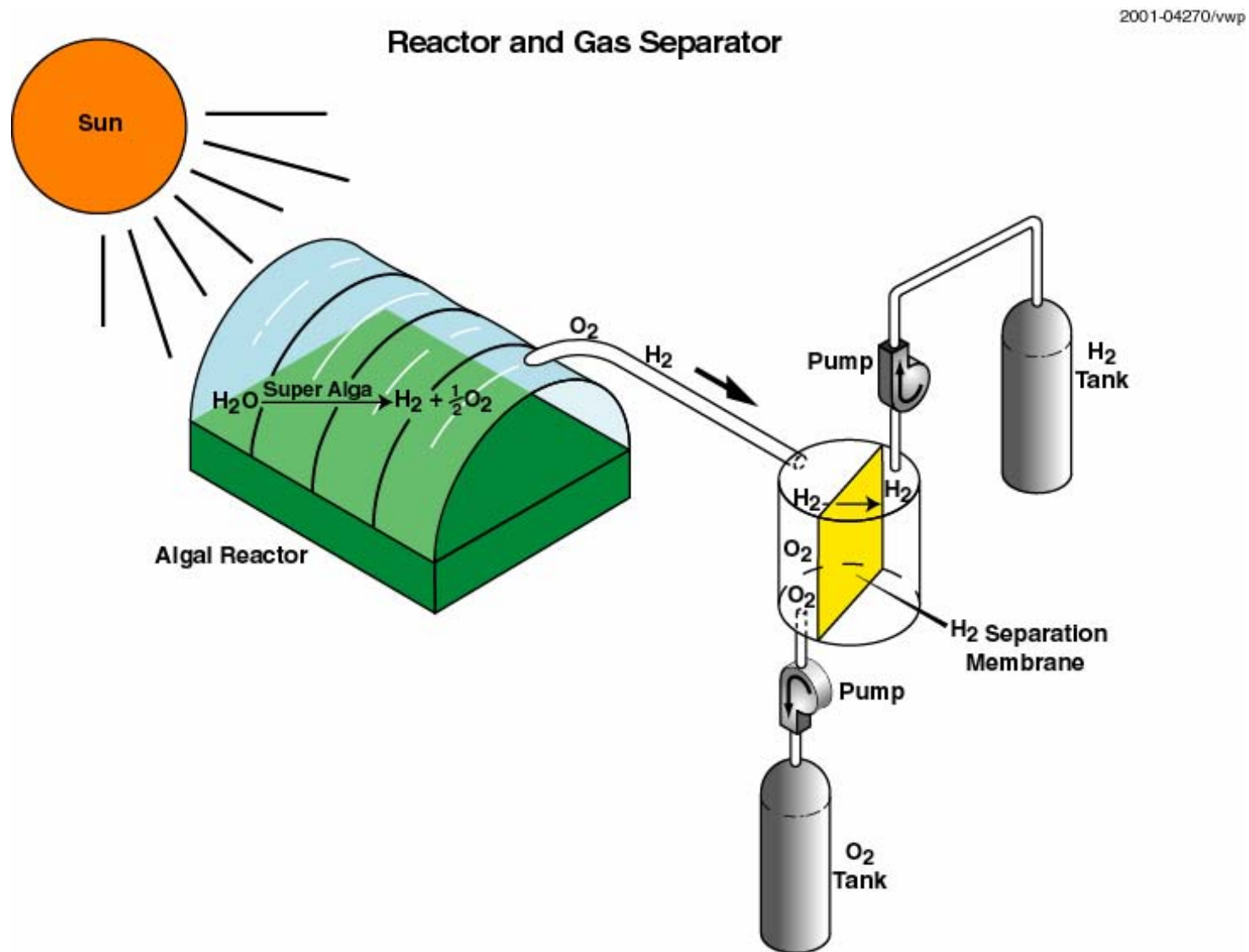
Year 1--Design and construction of DNA sequence coding for polypeptide proton channel (**accomplished for the first set of designer proton-channel genes**)

Year 2--Genetic transfer of hydrogenase promoter-linked polypeptide proton-channel DNA into DS521

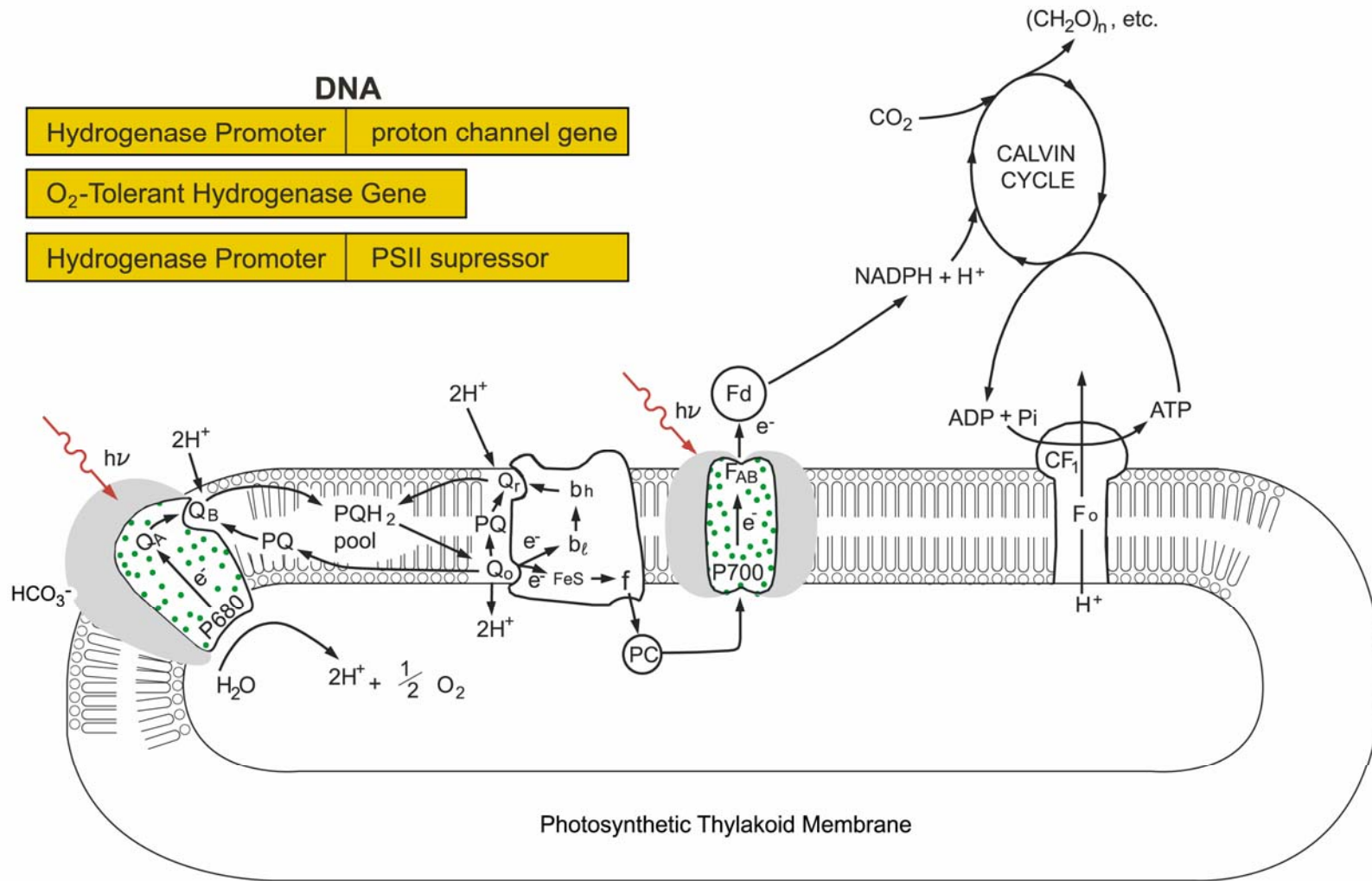
Year 3--Characterization and optimization of the polypeptide proton-channel gene expression

Year 4--Demonstration of efficient and robust production of H₂ in designer alga (ready for next phase: scale up and commercialization)

Our Envision (in Part B) How the Designer Alga will Be Used for Clean H₂ Production



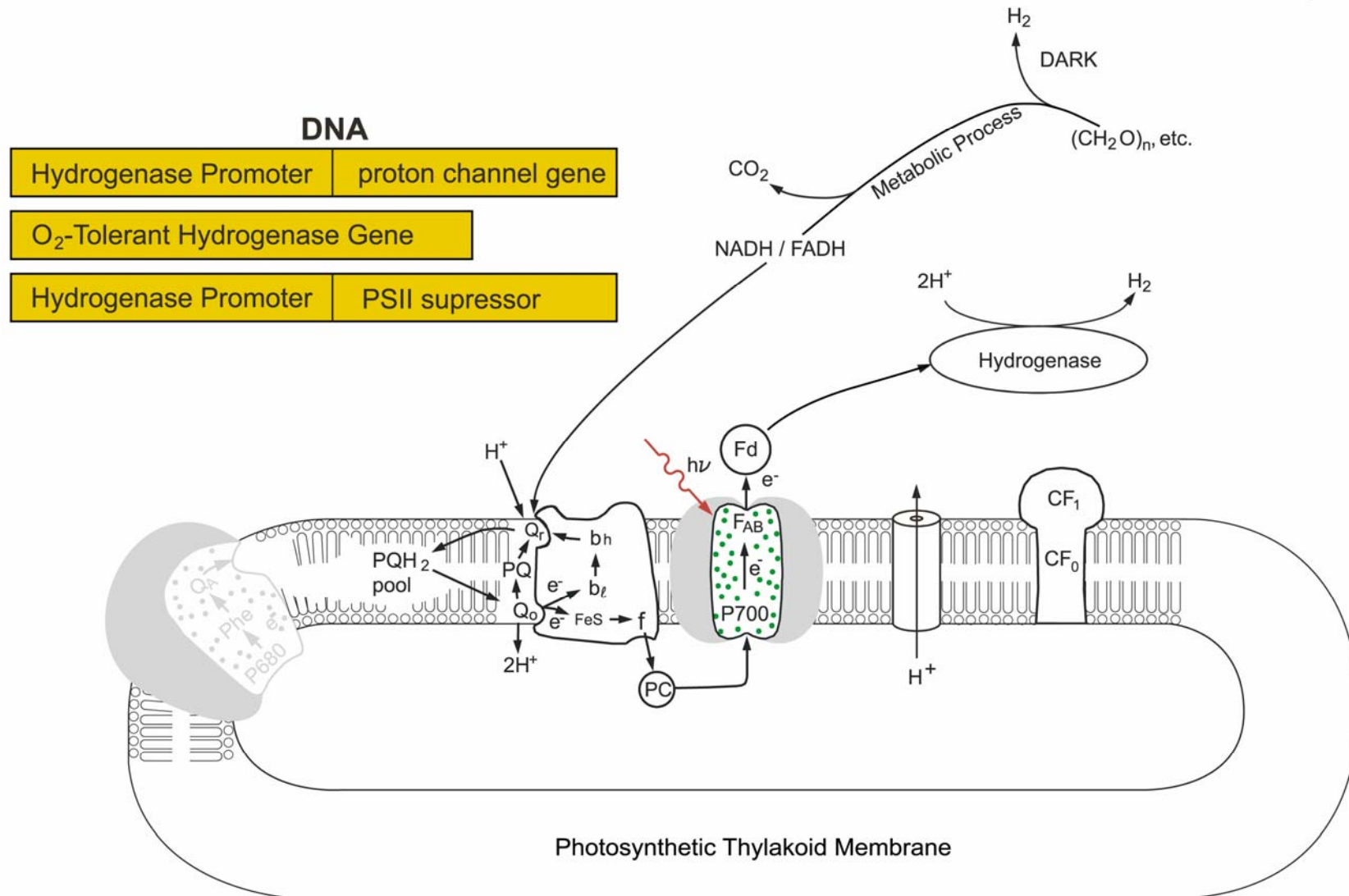
Designer Alga Upgraded with a Hydrogenase Promoter-Linked PSII Inhibitor (Suppressor)



ORNL 2002-04333/vwp

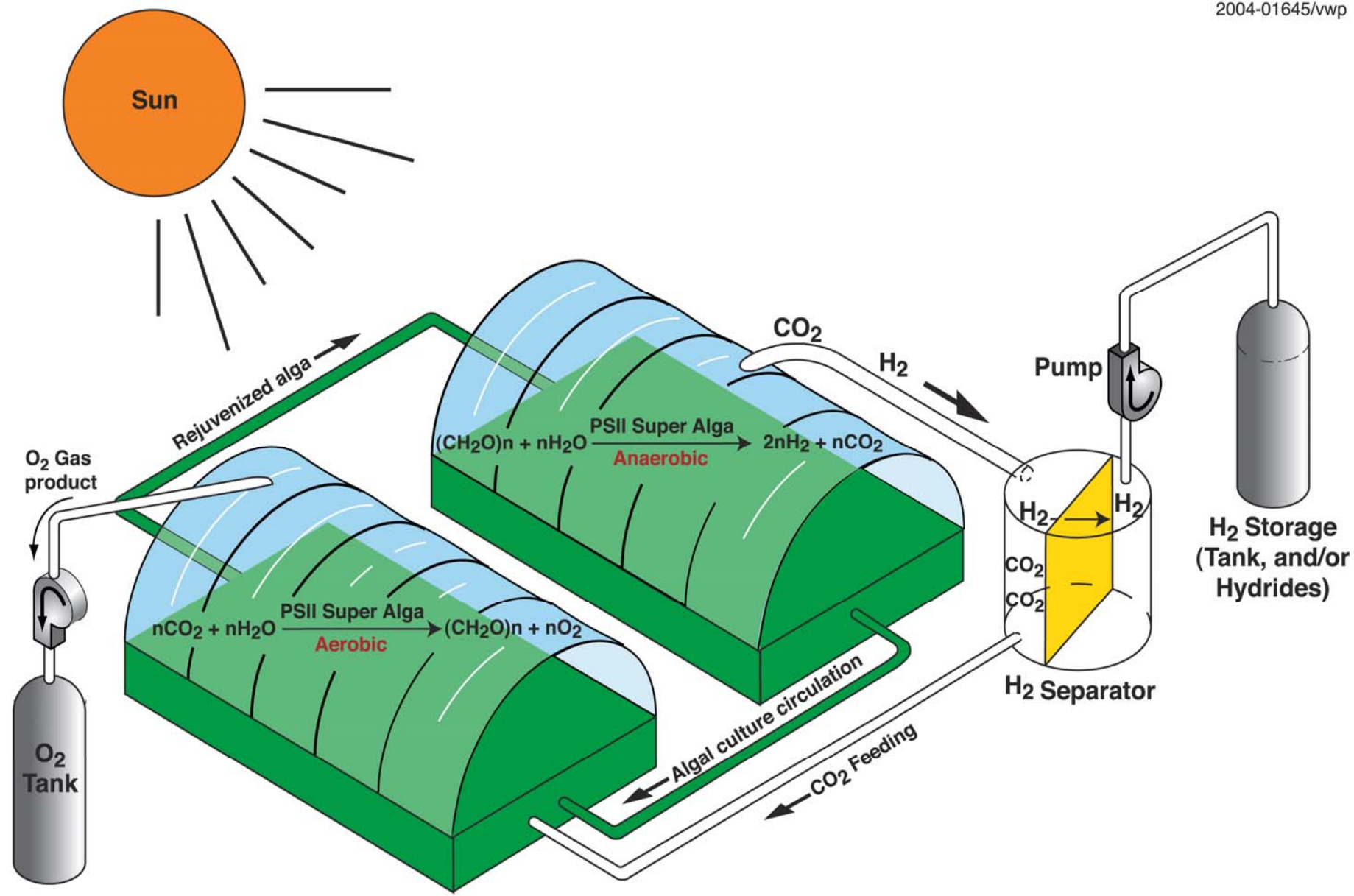
Expression of the Designed Genes for the PSII Inhibitor and the Polypeptide-Proton Channel under Anaerobic Conditions

ORNL 2002-04331/vwp



Envisioned Follow-on Bioreactor Development Project to Apply the Switchable PSII Designer Alga for Clean H₂ Production

2004-01645/vwp



Designer Alga H₂-Production Technology with 0.7% U.S. Land Could Provide H₂ Energy (30x10¹⁵ Btu) for All U.S. Cars

U.S. Total Land	U.S. Cropland	U.S. CRP (Set-aside land)	To produce 30x10 ¹⁵ Btu of H ₂ from H ₂ O by the Algal Technology
2,300 MM Acres	377 MM Acres	32.7 MM Acres	13.3 MM Acres
100%	16%	1.4%	0.6%

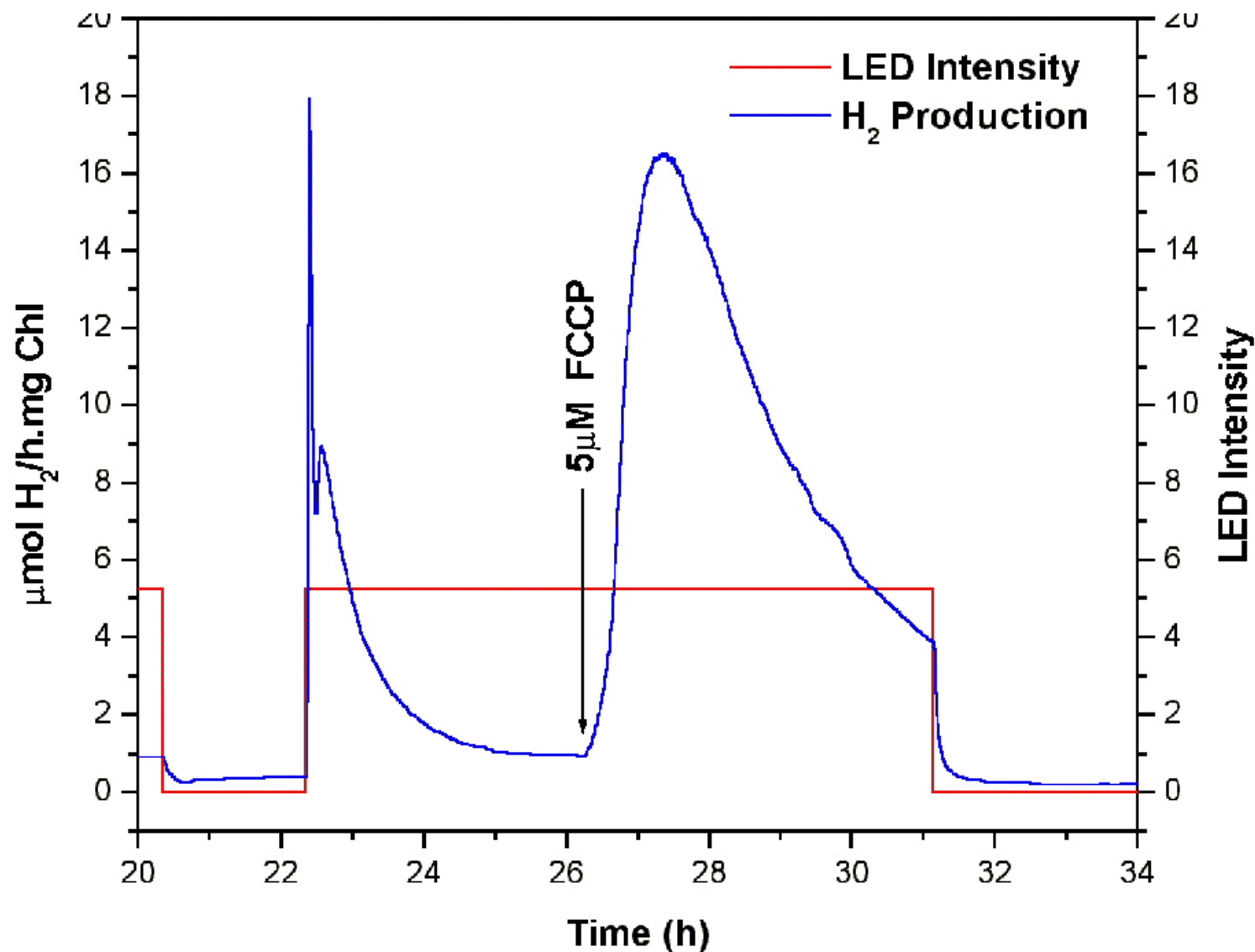
Calculated by Mark Downing and James Lee, using 1997 USDA NASS data and assuming 10% solar energy conversion efficiency for the Designer Alga H₂-production process

Designer Alga H₂-Production Technology Could Be an Attractive New Energy Business

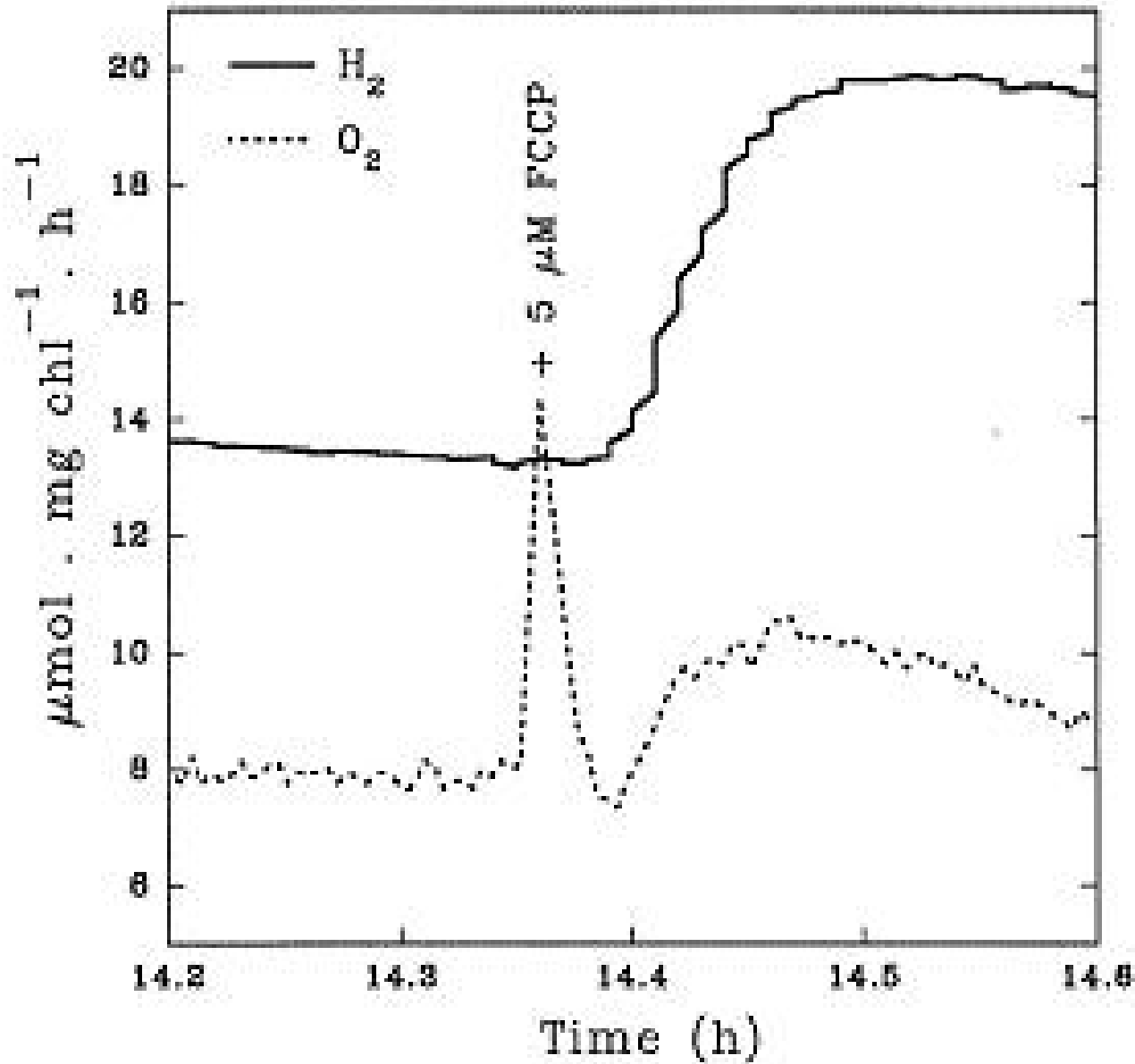
Designer-alga H₂ productivity	H₂ energy value produced	H₂ cash value at production site	Number of cars could be supported
21,519 Kg H₂/acre.year	2,419 MM Btu/acre.year	\$18,622/acre.year	140 cars/acre.year

Calculated by Dick Ziegler and James Lee, assuming the value of H₂ at production site will be \$1.00 per 115,400 Btu (equivalent to 1 gal of gasoline) and 10% solar energy conversion efficiency for the designer alga H₂-production process

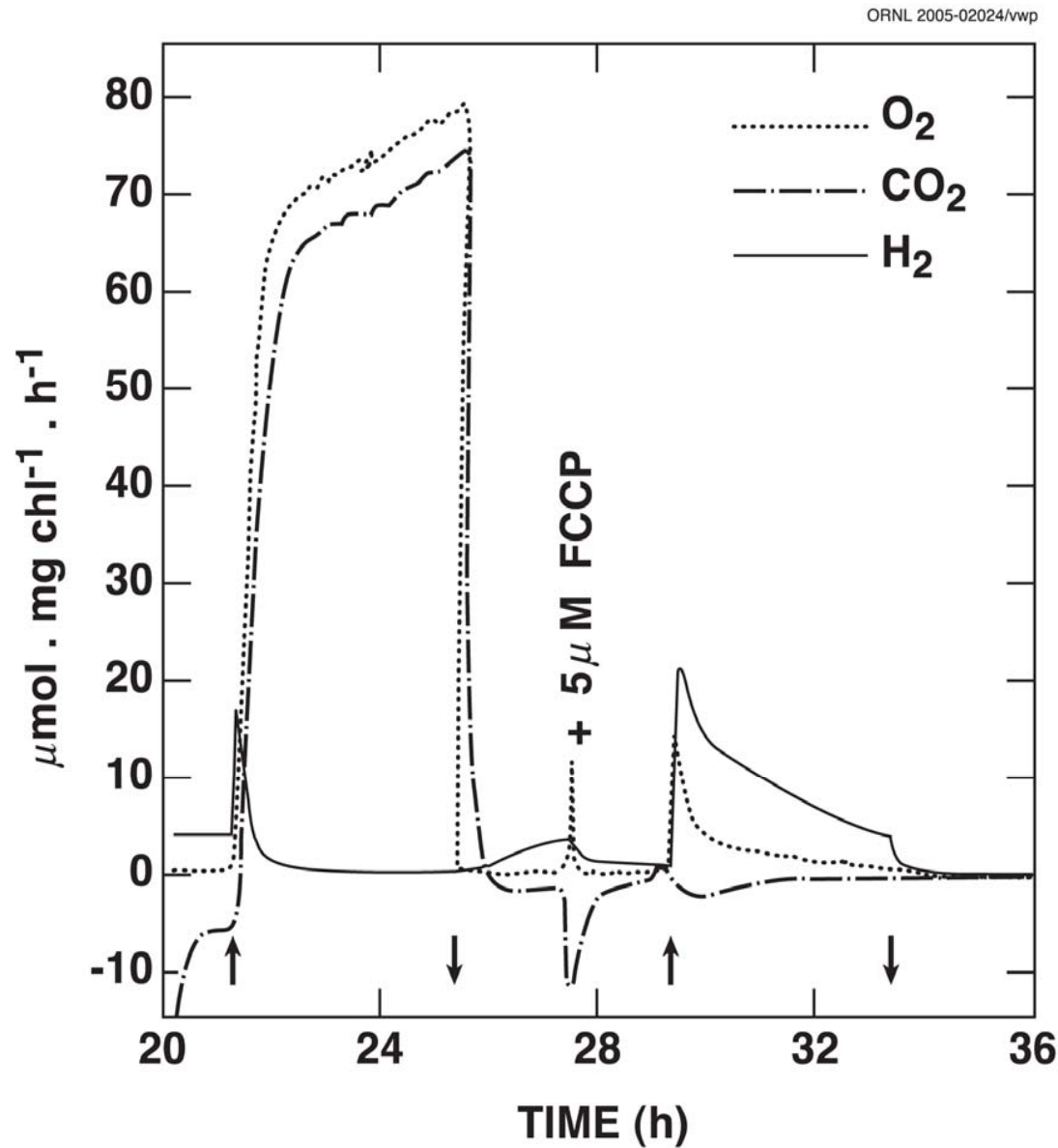
Proof of Principle Demonstrated by Proton Uncoupler FCCP Experiments in Wild-Type Algal H_2 Production with 1000 ppm O_2



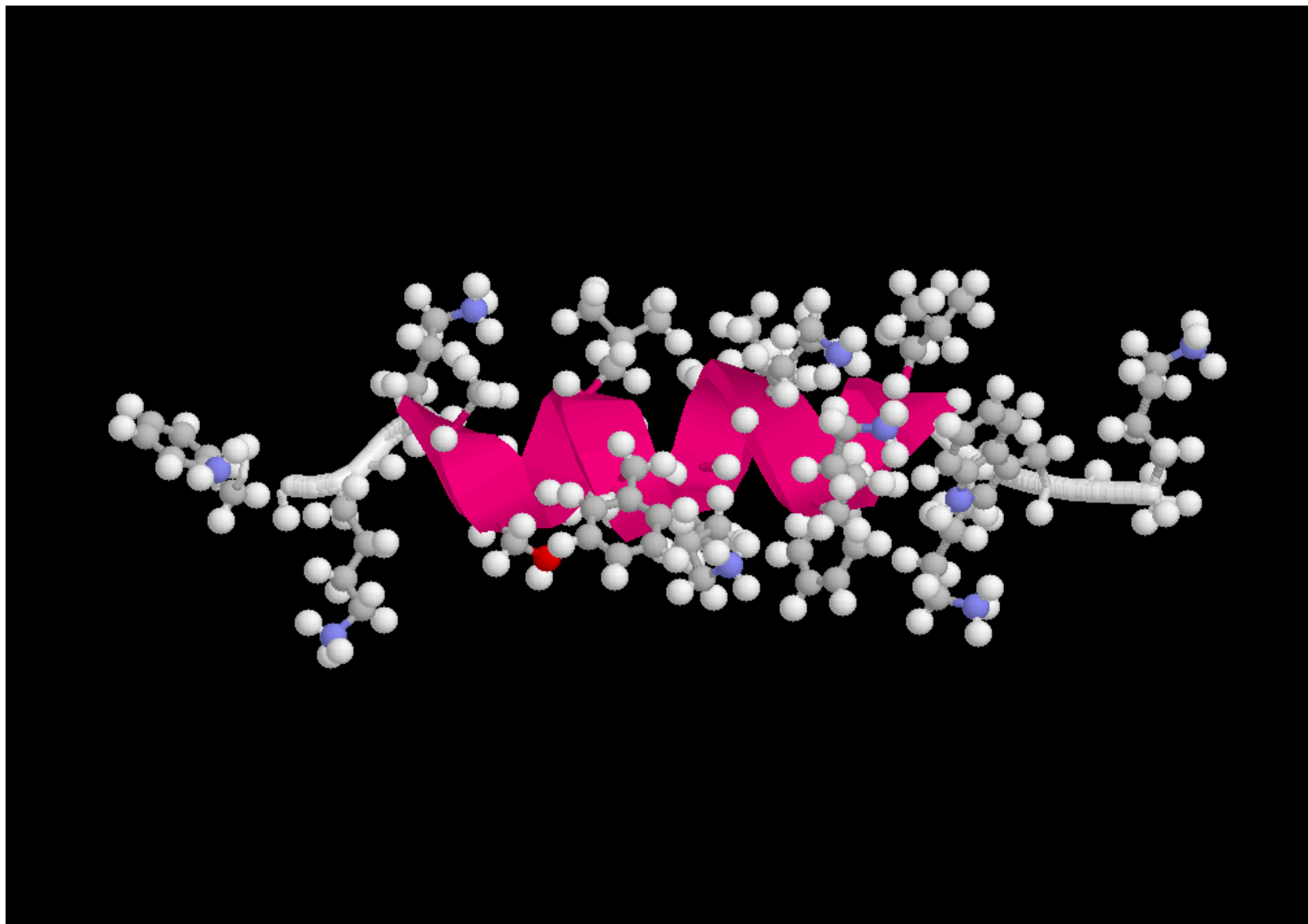
Proof of Principle Demonstrated by Proton Uncoupler FCCP Experiments in Wild-Type Algal H_2 and O_2 Production under Helium Atmosphere



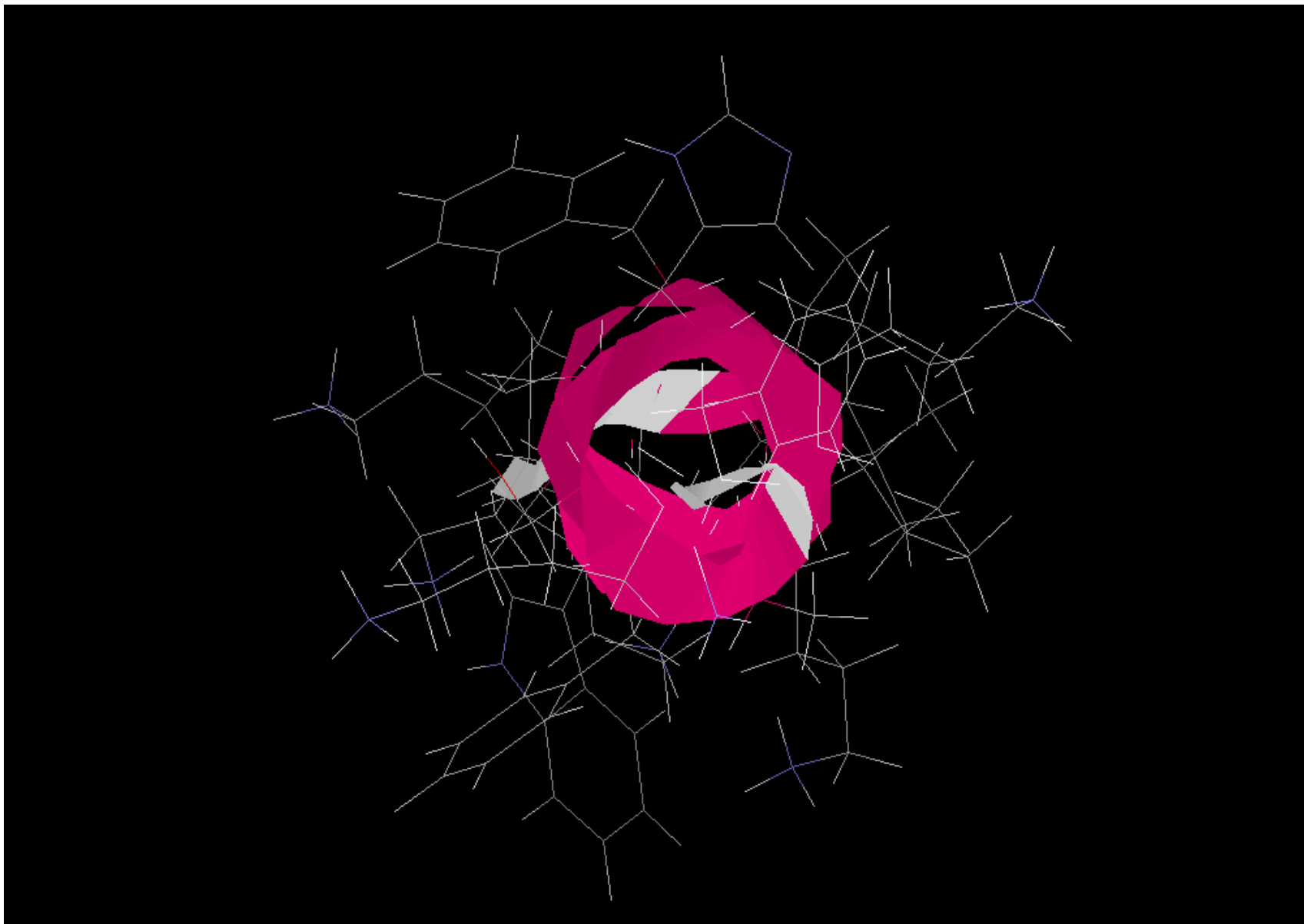
Proof of Principle Demonstrated by Proton Uncoupler FCCP Experiments in Wild-Type Algal H_2 and O_2 Production with 700 ppm CO_2 in Helium



Bioinformatics Analysis of Melittin Using the PROSECT Software



Top View of Melittin Structure Showing Its Channel Pore Size



Preliminary Results:

The Transformants will Be Screened and Cultured for a Number of Assays to Test for the Predicted Features of the Designer Alga

